DIFFERENTIAL RESPONSES TO MECHANOSTIMULATION IN EMBRYONIC STEM CELLS VERSUS THE EMBRYOID BODY MODEL OF DEVELOPMENT ASSESSED AT SINGLE CELL RNA-SEQ RESOLUTION

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Mechanical forces generated by gravity have shaped life on Earth and impact gene expression and morphogenesis during early development. In contrast disuse can reduce normal mechanical loading, resulting in altered cell and tissue function. Although loading in adult mammals is known to promote increased cell proliferation and differentiation, little is known about how cells respond to this stimulus during early development. In this study we sought to understand, with single cell RNA-sequencing resolution, how a 60-minute pulse of 50xg hypergravity-generated 5kPa hydrostatic pressure, influences transcriptomic regulation of developmental processes in the Embryoid Body (EB) model. Our study included both day-9 EBs and progenitor mouse embryonic stem cells (ESCs) with or without the hydrostatic pressure pulse. Single cell tSNE mapping shows limited transcriptome shifts in response to this pulse in either ESCs or EBs; this pulse, however, induces greater positional shifts in EB mapping compared to ESCs, indicating the influence of mechanotransduction is more pronounced in later states of cell commitment within the developmental program. We assessed ESCs and EBs for differentially expressed (DE) genes with hydrostatic pressure pulse and found approximately 1/3 DE genes were shared. However, gene ontology (GO) pathway analysis show that EBs have choreographed responses associated with upregulation of pathways for multicellular development, mechanical signal transduction, and DNA damage repair. Cluster transcriptome analysis of the EBs shows mechanostimulation promotes maintenance of transitory cell phenotypes in early development, including EB cluster co-expression of markers for progenitor, post-implant epiblast and primitive endoderm phenotypes versus expression exclusivity in the non-pulsed clusters. Pseudotime analysis identified three branching cell types susceptible to hydrostatic pressure induction of cell fate decisions. In summary, this study provides novel evidence that ESC maintenance and EB development can be regulated by mechanostimulation, and that stem cells committed to a differentiation program are more sensitive to force-induced changes to their transcriptome.

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mechanotransduction, embryonic stem cells, single cell RNA sequencing